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THE ROLE OF ECOTOXICOLOGICAL TESTING OF EFFLUENTS AND
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MacGregor, D.J.; Wells, P.G. 1984.

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**A Working Paper Prepared for the E.P.S. Laboratory Management Committee
(Dr. D. E. Thornton, Chairman)**



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November 1984

THE ROLE OF ECOTOXICOLOGICAL TESTING OF EFFLUENTS AND CHEMICALS IN THE ENVIRONMENTAL PROTECTION SERVICE

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SUMMARY

Ecotoxicological testing (primarily aquatic toxicity testing) of effluents, chemicals and environmental samples has an important role to play in support of toxic chemical programs and responsibilities of the Environmental Protection Service (E.P.S.). This paper summarizes the general roles of ecotoxicological tests, the history of applied aquatic toxicity testing in E.P.S., and the present capabilities and functions of the E.P.S. toxicity laboratories. The laboratories are making substantial contributions to a wide variety of E.P.S. and departmental programs on toxic substances.

EPS should maintain a strong operational capability and expertise for toxicity assessments compatible with the guidance of its strategic plan. Decisions are needed by the Laboratory Management Committee in specific areas to ensure efficient development and application of a flexible, integrated, hazard assessment scheme that encompasses both chemical and toxicological testing of effluents, chemicals and environmental samples. Eight specific types of aquatic toxicity tests are recommended for toxicity assessments in support of EPS and departmental programs on toxic chemicals.

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INTRODUCTION

This paper has been prepared to aid the Environmental Protection Service (EPS) Laboratory Management Committee and EPS senior management in an evaluation of the role of ecotoxicological testing in the Service's programs and laboratories. The policy framework within which this paper is written includes the DOE and EPS Strategic Plans, the Toxic Chemicals Management Program (TCMP) Strategic Plan, the Environmental Sensing Strategy (TCMP 1984), and agreements on the testing of chemicals at the international level by the Organization for Economic Co-operation and Development (OECD).

Chemical and ecotoxicological test data are required to evaluate the potential for environmental harm posed by a given effluent or chemical. This paper discusses the latter type of testing, in order to improve familiarity with the scope, strengths, weaknesses and applications of ecotoxicological testing within EPS.

This paper has five objectives:

- (1) To outline the general roles of ecotoxicological tests;
- (2) To summarize the history of aquatic toxicological testing in EPS laboratories;
- (3) To review the present capabilities and functions of the EPS toxicity laboratories;
- (4) To draw general conclusions and identify areas of concern on the future role of ecotoxicological testing in EPS;
- (5) To present recommendations on the continued conduct and application of specific aquatic toxicity tests, in support of EPS programs and responsibilities.

GENERAL ROLES OF ECOTOXICOLOGICAL TESTS

Ecotoxicology has been defined as being "concerned with the toxic effects of physical and chemical agents on living organisms, especially on populations and communities with defined ecosystems; it includes the transfer pathways of those agents and their interactions with the environment" (Butler 1978).

This section briefly describes how carefully chosen ecotoxicological tests contribute to hazard assessments* of effluents and chemicals, and how such tests relate to and are complementary with chemical approaches. The emphasis is on aquatic toxicological tests, as they are of primary value to EPS.

In general, hazard assessments and their component toxicity tests attempt to answer the question: Is this material (discharge or chemical(s)) harmful or likely to be harmful to the environment or to human health? This generates a series of secondary questions of environmental relevance such as the following:

- (1) What is the material of concern?
- (2) How much is being released, and at what frequency?
- (3) Is the material acutely toxic at the concentration at which it is released?
- (4) What factors affect the toxicity of the material, and what is the effect of treatment on the toxicity of discharges?
- (5) What is the likely environmental distribution of the material after release?
- (6) Is the material persistent, toxic and bioaccumulative after release to the environment, or is it changed to other materials which may be persistent, toxic, and bioaccumulated?
- (7) What is the nature of the sublethal adverse effects (reproductive, growth, developmental, behavioral, genotoxic, carcinogenic, etc.) of the material on biota, and at what concentrations do these occur?

Ecotoxicological testing attempts to provide answers to questions such as 3, 4, 6 and 7 above, which may pertain to effluents or chemicals. It should be noted that the ecotoxicological testing requirements will often differ for effluents (including leachates) compared to single chemicals; effluents are complex and variable mixtures with well-defined points of entry to aquatic environments, whereas single chemicals may be

* As defined by the American Society for Testing and Materials, ASTM STP 657. 1978.

released from many sources into a variety of media, and hence require general hazard assessments. Testing of effluents and chemicals can be both remedial and preventive.

Testing may also be used to address question 1 in the case of the discharge of complex mixtures. In such a case, the level of concern would be established by determining the source of toxicity in each mixture. One method of doing this involves a combination of physical/chemical fractionation, toxicity testing of the resultant fractions, and detailed chemical analysis of the most toxic of these (Samoliov et al. 1983). The underlying basis for the chemical monitoring of waste discharges is exposure assessment, which when linked to toxicological information identifies the concentrations of specific components that may be harmful to the environment.

From the perspective of the non-specialist, a great many ecotoxicological tests exist (note Bitton 1983, Moriarty 1983, Persoone et al. 1984, Connell and Miller 1984, Rand and Petrocelli 1985, among others). Many tests are being refined or developed. However, all of these tests are designed to fit into an evaluative framework such as that outlined above. The specific test or tests which should be employed by an agency are directly related to its responsibilities and to the level of hazard assessment and environmental protection which is required. If the level of protection corresponds to the prevention of acute lethality to fish, acute lethality testing with one or more representative fish species would be appropriate. Surrogate test organisms (for e.g., bacteria) may be employed in place of representative species for the rapid preliminary toxicity screening of discharges, process streams or chemicals. At a higher level of desired protection, such as prevention of adverse chronic effects in populations of several species, the scope of the testing would be extended to include various types of sublethal responses, as well as to effects on lower trophic levels (the food supply). Field observations of the species of interest and of its habitat would be another essential component at this level of activity. Fortunately, because of considerable interagency and international activity in ecotoxicological testing, a substantial resource of skills, methods and data is available. Hence, it is unlikely that any single agency would be required to develop all of the appropriate information independently in order to conduct hazard assessments of pollution problems.

Aquatic toxicity tests on effluents and chemicals (alone and in mixtures) are usually conducted concurrently with chemical analyses in order to describe the material(s) and concentration(s) causing the toxicity. As stated above, this combination usually provides more interpretable information than either type of analysis alone. However, as recently reemphasized (Hanmer and Newton 1984), toxicity tests on complex effluents and

environmental samples overcome several of the limitations of chemical testing. The biological effect of mixtures and the influence of multiple factors is addressed, many toxicity tests can be conducted rapidly and inexpensively, and dischargers find toxicity data interpretable and credible. Hence toxicity is a very useful parameter in its own right to measure, particularly for effluents, because it is meaningful and it can lead rapidly to positive action to reduce or eliminate a pollution problem.

Ecotoxicity is also an important component of the series of test guidelines developed by the OECD for the assessment of chemicals. Guidelines developed for this purpose to date include protocols for measuring growth inhibition in algae, reproduction in aquatic invertebrates, and acute toxicity in fish. Further guidelines will be available soon for other aspects of toxicity in fish, as well as for birds, earthworms, terrestrial plants and microorganisms in activated sludge. A closely related series of OECD guidelines is also available for the evaluation of bioaccumulation and biodegradation of organic chemicals. A recent initiative by OECD will result in a set of guidelines on the use of biological tests on industrial and municipal effluents, and receiving waters (Environment Canada 1984).

There is implicit support of ecotoxicological testing within EPS. The Strategic Plan (Anon., 1982) calls for the provision of "information on the impacts of toxic chemicals," and for the initiation of "a systematic approach to the development of information necessary for the control of existing toxic chemicals which are a threat to the environment." In a similar vein, the TCMP Strategic Plan (Anon., 1983) notes the importance of developing and maintaining "a scientific cadre capable of detecting and evaluating toxic chemicals throughout the environment," and of the "identification of selected species and specific bioeffects as biological indicators of toxic chemicals impact to be used for ecotoxicity testing." Ecotoxicological testing is considered an important component of the knowledge-base in the long-term DOE environmental sensing program thrust (TCMP 1984).

HISTORICAL OVERVIEW

There has been a number of documents describing the status and role of toxicity testing to achieve the primary EPS objectives of protecting and enhancing environmental quality by preventing or reducing pollution.

During the 1970's, the control of industrial effluents in Canada by E.P.S. was mainly based on the use of a single acute toxicity test (the rainbow trout lethality test) and a basic knowledge of the chemical composition of effluents and the major constituents contributing to toxicity. This was the first approach to controlling major problems. A number of industrial effluents were controlled to specified chemical and toxicity limits, stipulated in guidelines and regulations under the Fisheries Act. The regional EPS laboratories were assigned responsibilities for investigating specific effluents, identifying acceptable limits for discharges, conducting compliance monitoring tests and legal tests, as well as supporting studies of selected regional problems. Most toxicity studies were conducted with the trout test. During the period, several laboratories also recognized the usefulness of water flea (*Daphnia*) acute tests, among others, and applied them whenever required. Application of acute toxicity tests on effluents is continuing, as a first level of control, but this application has had decreased support in recent years (Dafoe *et al.* 1984).

A paper by Pessah and Cornwall (1980) described the role of the rainbow trout acute lethality test in monitoring effluents, setting and enforcing effluent standards, and investigating the toxicities of specific chemicals and formulations. The rainbow trout test was developed as a standard protocol for EPS (Anon. 1980a). The EPS trout toxicity protocol has been used as a source document for two provincial documents - B.C.'s Provincial Guidelines and Laboratory Procedures for Measuring Acute Lethal Toxicity of Liquid Effluents to Fish, and Ontario's Protocol to Determine the Acute Lethality of Liquid Effluents to Fish. The trout test is also incorporated in the revised Canadian Dispersant Guidelines (EPS 1984). It is supported by a large base of knowledge (Wells and Moyle 1981).

Environmental concern and responsibilities in E.P.S. shifted by the late 1970's to individual waste streams, non-lethal final effluents, contaminated sediments, individual classes of substances, new chemicals, and new formulations. Studies were conducted of toxicity reduction across treatment systems (Cairns *et al.* 1979). The sublethal effects of treated effluents to a variety of organisms were evaluated (Sprague *et al.* 1978, Westlake *et al.* 1983a, b). Dredged sediments from harbors were tested using a bioaccumulation

protocol with an intertidal clam (Sharp 1977), in support of the Ocean Dumping Control Act. A report prepared by the EPS Aquatic Toxicology Group (Anon. 1980b) illustrated the desire to better utilize its expertise, facilities and toxicity procedures for the hazard assessment of existing chemicals and effluents. The Workshop on Biological Testing and Hazard Assessment (Blaise 1984), held in October 1982, produced several summaries of the available ecotoxicological tests and their potential applications for EPS purposes. One of the papers (Brydon 1982) differentiated between the needs for new chemical toxicity assessments, and assessments of effluents and their constituents, the author supporting the latter as an EPS operational activity requiring a toxicological capability. The Intergovernmental Aquatic Toxicity Group (IGATG), established in 1976, meets annually to discuss technical details of toxicity tests, the selection and application of new tests, and the conduct of intra/inter-laboratory calibration exercises. They, for example, prepared the above mentioned trout protocol (Anon 1980a). The members of IGATG maintain a knowledge of current ecotoxicity testing procedures and their applications for screening chemicals, and testing effluents, sediments and various surface waters for acute and chronic effects. Various individuals in EPS are active in the annual Canadian Aquatic Toxicity Workshops, for which the Department of Environment (DOE) and the Department of Fisheries and Oceans (DFO) are co-sponsors. As stated earlier the Toxic Chemicals Management Program is preparing the strategy for an integrated program on environmental sensing, which includes the application of various ecotoxicological methods at all stages of the management of toxic chemicals (TCMP 1984).

For several years, the real and perceived difficulties of interpreting the results of ecotoxicity tests other than lethal tests, and applying the results in decision-making, have somewhat limited their use for EPS purposes. However, the benefits of current ecotoxicology and sensitive sublethal tests are becoming more obvious. The limits of chemical analysis, especially with mixtures, are being reached; many substances are present in very low but potentially harmful sublethal concentrations; and the interpretive problems with chemical data are being realized (Environment Canada 1984). There will be a continued high level of research activity in environmental chemistry, environmental toxicology and hazard assessment, particularly in North America and Europe. However, in 1984, sufficient knowledge and numbers of practical biological tests exist so as to augment their current status and role in EPS.

TESTING CAPABILITIES AND APPLICATIONS WITHIN EPS

It may be advantageous to introduce this section with a general description of the different types of aquatic toxicity tests. The choice of test depends upon the question(s) being asked and the level of confidence required of the answer(s). The tests chosen ultimately depend upon the type and progression of the hazard assessment chosen for the specific effluent or chemical.

Four general categories of tests, adopted from Wells (1984b), range from the simplest laboratory test with a large degree of precision and few variables to the in-situ tests with less precision and many more sources of variation. The tests are as follows:

- 1) Acute lethal and sublethal toxicity tests in the laboratory (Screening tests). These tests provide basic information on the toxicity of a material, or data on the comparative toxicity of several materials. They are essentially "screening" tests. They may last minutes or days. They are often static, with single end-points in single species to measure, and hence are relatively "simple" tests. Multiple species tests for screening are in an early stage of development, but show great promise. Cost per toxicity test is relatively low (e.g. \$200-\$500 for fish tests depending upon the number of tests run simultaneously). Precision is high, but realism (representativeness of field conditions) is often low. These tests can be used to determine the effects of major variables on the toxicity of materials. With the correct interpretation, these acute tests are a very powerful tool in the hazard assessment process.
- 2) Chronic sublethal tests in the laboratory are used to determine the effects of longer, low-level exposures on key properties of individuals and their populations. They are applied when the tested materials are persistent, bioaccumulative, and toxic in the acute tests. The tests may be several weeks or months in duration, depending upon the type of test organism. Complexity and costs are greater; often semi-static or continuous - flow modes of exposure are used and many measurements are taken daily. The cost per trial per chemical or effluent could be as high as $\$5-10 \times 10^3$ dollars, excluding expensive and detailed analytical chemistry. The precision, with skilled personnel, can be $\pm 50\%$ in the same laboratory. The exposure and other test conditions more closely represent field conditions - concentrations simulate those in the field and the effects of major variables on chronic toxicity (body size, season, reproductive condition, etc.) can be studied.

- 3) Microcosm and mesocosm tests are one to several months in duration, and have as their main objective the measurement of population processes and responses (diversity and abundance changes, shifts in predator-prey relationships, physiological-biochemical responses of specific populations) when exposed to the introduced material(s). Field mesocosm tests are complex to conduct, requiring significant investments of materials, skills and time. The complexity and costs ($>10^5$ dollars/material) can be reduced with the selection of simpler laboratory-based microcosm tests. The repeatability of the large-scale outdoor tests is low, but measures of impact and its variability are probably more realistic.
- 4) Acute or chronic in-situ tests. These tests can be sensitive, realistic tests of water quality at selected sites and can be linked to other "effects monitoring" approaches, such as benthic surveys. Some tests have been used by EPS in past years (Parker and Doe 1981). Considerable international efforts are occurring to develop suites of practical, sensitive, and interpretable in-situ toxicity techniques (McIntyre and Pearce 1980, Mount *et al.* 1984, Environment Canada 1984).

The selection of tests for a particular problem also depends on technical considerations such as the limits of reliable measurements and the precision, sensitivity and simplicity of the test. In the early days, fish tests were chosen for biological as well as practical and legal reasons. A wider choice of organisms and approaches is available now (Persoone *et al.* 1984). However, all tests have limitations. Prediction of all possible environmental hazards for many materials cannot be expected. Any chosen testing scheme, with one or more tests, will only contribute to a correct assessment.

The aquatic toxicity tests which are conducted in the EPS laboratories across Canada as of May 1984 are summarized in Table 1. They include freshwater and marine, acute and chronic, and lethal and sublethal tests, covering a wide range of responses (Tables 1 and 2) and applications (Table 3). The main features of each of the tests are presented in detail in Appendix A.

The most frequently used organisms, and ones for which there are well developed facilities, tests and skills, are the trout, water flea (cladoceran), algal, bacterial (e.g. Microtox), and marine fish. The trout, water flea and Microtox tests are used in 4 of 4, 3 of 4 and 3 of 4 regional laboratories, respectively. Less of a capability exists with the algal and saltwater fish tests, except in specific regions. A wide range of other tests is available, primarily used for effluent evaluations and specific studies, such as on contaminated sediments and dredging spoils (Tables 1-3). The coordinated

development of several tests between laboratories, and the adoption and/or development of other procedures, reflects the commitment of the staff to devise and apply toxicity test procedures in a large number of EPS and DOE programs.

The applications of the various tests used in the laboratories are numerous (Table 3) and vary greatly from region to region. The following summary is based on reports given on each region's activities at the November, 1984, meeting of the Intergovernmental Aquatic Toxicity Group (IGATG) in Vancouver, B.C. There are four main laboratories, in Vancouver, Edmonton, Longueil and Dartmouth, with smaller facilities in Whitehorse and St. John's.

The laboratory for EPS, Pacific, is a service laboratory, serving primarily EPS, B.C. Province, DIAND, and DFO. Most tests are with rainbow trout, with some use of the Microtox procedure and the three-spine stickleback (Table 1). The fish tests are acute lethal ones. The laboratory tests effluents, particularly those from the pulp and paper and mining industries. The laboratory is involved in legal tests for impending prosecutions, and in the hazard assessment of spills and their cleanup activities. There is also a small bioassay facility at Whitehorse, using Daphnia tests (Table 1) in investigations of sewage and mining effluents, as well as specific chemicals such as anionic flocculants from placer mining. A total of 3.2 PY's is used in this work, as well as 1 PY from the B.C. government.

The laboratory in Edmonton provides a service for EPS, the Canadian Wildlife Service (CWS), the provinces and some municipalities. Several organisms and test procedures are used (Table 1). The rainbow trout procedure is used infrequently, the Daphnia and Microtox tests being most useful. The laboratory conducts studies on oils and oil dispersants, land-fill leachates, specific chemicals, pesticides and ambient waters, all in support of regional programs. Personnel include 3 PY's and 1 contract worker.

The laboratory at Longueil, P.Q., has the capability to apply a wide range of tests, particularly small-scale, rapid, sublethal tests organized for hazard assessments (Table 1, Blaise *et al.* 1984). This laboratory is very progressive in its use and refinement of procedures with algae, protozoa and bacteria. Most tested samples are industrial and municipal effluents, surface waters, and contaminated sediments, the tests being conducted for several EPS and DOE programs. About 3 PY's is dedicated to this work.

The laboratory at Dartmouth, N.S., conducts a wide variety of tests in support of EPS programs (Tables 1-3). The laboratory makes extensive use of rainbow trout for industrial compliance tests, primarily on effluents from the pulp and paper, refinery and mining industries. The laboratory is involved in many programs for EPS and other

government groups - contributing to the data base for the Code of Good Practice for Gold Mining; screening the toxicity of drilling muds and their components for the Canada Oil and Gas Lands Administration (COGLA); coordinating and developing the toxicity protocols for oil rig drilling cuttings and drilling muds; developing and applying behavioral and bioaccumulation tests with marine clams; and investigating factors such as pH which influence the toxicity of pesticide formulations. In addition, this laboratory has organized and coordinated an EPS interlaboratory calibration study with Daphnia, of importance both to EPS and to OECD. A total of 3 PY's is committed to this work.

The above descriptions show that the EPS toxicity laboratories have the capacity to perform a number of toxicity tests in support of a variety of objectives, programs and intervention activities worked on by the Service. This capacity is relatively flexible within practical limits. The application of this capacity varies significantly from region to region in Canada, i.e. regulatory and legal tests (EPS Pacific and Yukon), hazard assessment of effluents and receiving waters (EPS Quebec) and effluent monitoring and many specific marine problems (EPS Atlantic). The laboratories apply well-recognized, practical, cost-effective and reliable tests in support of a very wide range of EPS programs and programs of interest to EPS. In some cases, single tests alone are performing a valuable function in a program, whereas in other situations (e.g. The St. Lawrence River), batteries of different tests are applied. Above all else, all regions of EPS are making extensive use of their in-house testing capability.

Note: This section could be expanded to include a more detailed and critical examination of ecotoxicological testing in EPS in relation to its program activities and priorities (i.e. programs, results, clients, resources and resource recovery). In addition, the data in Appendix A could be evaluated in support of the quality control of the most common testing protocols. Both tasks would be most capably done by the EPS members of IGATG.

CONCLUSIONS

The role of an applied ecotoxicological testing capability within EPS is evident, and is supported by the EPS Strategic Plan. Such a capability allows for testing in support of several statutes (Fisheries Act, Ocean Dumping Control Act, Environmental Contaminants Act) administered by DOE. It often identifies problems, particularly with effluents and other mixtures, prior to detailed chemical analyses. This capability allows EPS to contribute to competent and current hazard assessments on various types and combinations of toxic substances from a variety of sources. It also contributes to maintaining experienced personnel (in ecotoxicology and environmental chemistry) who are critical at various stages of the problem assessment process, the presentation of expert testimony in court, and very important to the quality control of internal and contracted work. A strong ecotoxicological testing capability in EPS assists in defining and resolving many current problems, and contributes to avoiding costly and damaging future problems caused by toxic substances in waters, sediments and biota.

From a scientific viewpoint, acute lethal and sublethal laboratory toxicity tests for screening purposes are well recognized and established. Advances in chronic sublethal laboratory tests are rapidly making these tests more applicable. More R & D needs to be conducted before EPS can adopt approaches with microcosms, mesocosms and in-situ tests for routine applications, but an awareness of such procedures can contribute to the problem identification and assessment functions.

In 1983 the resources expended for toxicity testing amounted to \$2.1 million while \$4.7 million was spent on chemical/physical monitoring. These figures, which require verification, indicate a 30 - 70 split between the two. The present level of resource allocation, particularly with PY's, to do assigned tasks in ecotoxicology is at a minimum level. There should be a concerted effort to discuss, develop and apply an integrated hazard assessment scheme encompassing chemical and toxicity testing in a mutually supportive fashion. This will maximize the use of limited resources in the laboratories.

The sections below are areas of concern where specific policies and objectives need to be developed. An agreement to address these areas of concern is required before detailed recommendations are developed.

1. Mandate

The type of ecotoxicological testing to be applied in a given instance depends on the required level of hazard assessment. Hence, it is incumbent upon EPS management

to decide if more than the limited preliminary screening capability of effluents and chemicals which now exists will be required in the future. That is, EPS should decide upon the level of protection it wishes to provide to the environment from its intramural ecotoxicological capability and how it can best use this capability. A more thorough review of the strengths and shortcomings of the existing EPS toxicity capability in relation to EPS responsibilities and programs, coordinated by the Laboratory Management Committee, would assist in this decision.

2. Adequacy of present resources

Present resources are barely adequate to meet differing regional operational requirements, but are inadequate for the purposes outlined in recent Strategic Plans, for chemical testing in accord with the OECD testing guidelines, or for systematic input of new data and data summaries into Service hazard assessments. In addition, the level of technical facilities available in support of specific programs such as Ocean Dumping requires upgrading, particularly with regard to the quality assurance of contracted work and the role of the laboratories as reference laboratories for specific protocols and programs of EPS and OECD.

3. Central Coordination

The Laboratory Management Committee should be one mechanism for involving the regional laboratories and IGATG in the ecotoxicological aspects of new programs and program development.

4. Interlaboratory Collaboration and Cooperation

Given the available level of support and the diversity of toxicological testing activity in the various laboratories, the IGATG has done an excellent job. Its continuing activity should be encouraged, its function defined, and its connection to laboratory and program management established. Activity along these lines has been recently initiated by the Laboratory Management Committee (Oct./84).

5. Extent of Testing

Even rudimentary hazard assessments make provision for ecotoxicological testing in aquatic, terrestrial, and avian biota, and this ecosystem approach is implicitly recognized in our Strategic Plans. Only aquatic testing has been performed to date in EPS laboratories, and decisions are needed on the extent of future testing, and the mechanisms for continued collaboration and coordination with ECS programs and laboratories.

RECOMMENDATIONS

The function of the EPS toxicity laboratories is to provide technical support for departmental programs designed to prevent irreversible acute or chronic effects on aquatic biota as a result of human activities. From the ecotoxicological perspective, a series of tests is needed which incorporate several representative species and biological responses that account for the varied sensitivities of different trophic levels and life stages. These tests can be applied in a batch or tiered manner. We recommend the maintenance of a capability to conduct the following specific aquatic toxicity tests:

1. Lethal and sublethal tests for freshwater and marine fish (rainbow trout and three-spine stickleback). Only lethal tests are widely employed with these organisms in EPS laboratories at present.
2. Growth inhibition tests in algae (Selenastrum capricornutum). This test is routinely employed only in the Quebec Regional Laboratory.
3. Lethal and reproductive tests in zooplankton (Daphnia magna). These tests are now employed in some EPS laboratories.
4. A sublethal toxicity test in bacteria (Photobacterium phosphoreum, Microtox^R test). This test has a rapid screening and predictive capability but is best employed in concert with one or more of the above tests. It is available in the Québec and Western and Northern Regions.
5. Lethal and sublethal tests with young life history stages of other species of importance (e.g. crustacean larvae, fish embryos and larvae, etc.). These tests are employed because young life stages are generally more sensitive to contaminants than the corresponding juveniles and adults. EPS laboratories have only limited experience with these important and widely applicable tests.
6. Bioaccumulation/toxicity tests. These tests determine the extent to which chemical constituents of effluents and contaminated sediments accumulate in aquatic biota. Such accumulation may result in short-term or long-term adverse effects in such biota and/or contamination of the human food chain. Only one such test has been employed formally to date, but there is a much wider requirement for such tests, especially in association with the Ocean Dumping Control Act.
7. Field tests. These are employed to verify the results of the above laboratory tests under more environmentally realistic conditions. Typical field tests include the use of caged test organisms in the vicinity of discharges, the measurement of

contaminants bioaccumulated in selected natural species, and studies of community viability and diversity. These tests have not been widely employed by EPS laboratories to date.

8. OECD tests for specific chemicals. These tests comprise a wider series of tiered tests, which address physical properties, biodegradation, human health effects, and ecotoxicological tests (including most of those noted above). Development of the capability to perform selected OECD tests in some EPS laboratories is in progress.

The best utilization of a selection of the above tests is within the framework of a flexible, interdisciplinary hazard assessment scheme specifically addressing the programs and problems at hand. An organized and well-supported approach to utilizing specific tests would give the Service the best available ecotoxicological capability in support of our mandate for the foreseeable future.

Implementation of these recommendations, totally or in part, in support of EPS responsibilities and legislated mandate should be considered as soon as possible, following a detailed review of this report by the Laboratory Management Committee.

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TABLE 1 ECOTOXICOLOGICAL TESTS WITH AQUATIC ORGANISMS, CONDUCTED IN ENVIRONMENTAL PROTECTION SERVICE LABORATORIES, CANADA, AS OF MAY 1984

Description of Test		Regional Capability ¹ , 1986					
Medium and Type	Species	Responses	General Applications of Test	P	W	Q	A
FRESHWATER							
Acute, lethal	1. Green algae (<i>Selenastrum capricornutum</i>)	- Mortality	- screening - selected investigations			O ²	
	2. Water flea (<i>Daphnia magna</i> , <i>D. pulex</i>)	- Mortality - Incapacitation	- screening of effluents, chemicals, environmental extracts, formulations - selected investigations	●	●		
	3. Rainbow trout (<i>Salmo gairdneri</i>)	- Mortality - Incapacitation	- regulatory monitoring - screening of effluents, chemicals, environmental extracts - legal testing - selected investigations	●	●		●
	1. Ames test (<i>Salmonella</i> sp.)	- Reverse mutation (mutagenicity)	- screening of mutagenic/carcinogenic potential of effluents, chemicals, environmental extracts		○		●
	2. Microtox system (<i>Photobacterium phosphoreum</i>)	- Inhibition of light emissions	- screening effluents, chemicals - selected investigations	●	●		●
Chronic, sublethal	3. Green algae (<i>Selenastrum capricornutum</i>)	- Inhibition of population growth - Physiological stress (ATP)	- screening effluents, chemicals, formulations - selected investigations		○		○
	4. Ciliate Protozoan (<i>Colpidium campyllum</i>)	- Inhibition to ingest bacterial food source	- screening effluents, chemicals - selected investigations				○
	5. Rainbow trout (<i>Salmo gairdneri</i>)	- Physiological stress (ATP, four days) - Bioaccumulation	- selected investigations				○
	1. Algae-microtox	- Bioavailability and transfer of toxicants	- predictive bioaccumulation potential for effluents and chemicals - selected investigations				●
	2. Water flea (<i>Daphnia magna</i> , <i>D. pulex</i>)	- Inhibition or delay of reproduction - Brood size, mortality - Life cycle effects	- screening effluents and chemicals - testing of commercial chemicals			○	
SEAWATER	3. Rainbow trout (<i>Salmo gairdneri</i>)	- Physiological stress (ATP, 21 days) - Long-term exposure and mortality - Hatching success, survival and growth of larvae	- selected investigations - testing of selected chemicals, effluents, sediments, land-fill leachates, environmental fractions - selected investigations			○	○
	1. Brine shrimp (<i>Artemia nauplii</i>)	- Mortality - Incapacitation	- screening effluents and chemicals				○
	2. Clams, mussels	- Incapacitation	- selected investigations				○
	3. Three-spine stickleback (<i>Gasterosteus aculeatus</i>) and other marine fish	- Mortality - Incapacitation	- screening and monitoring effluents - screening chemicals - selected investigations	○	○		
	1. Microtox system (<i>Photobacterium phosphoreum</i>)	- Inhibition of light responses	- screening, monitoring effluents - screening of chemicals - selected investigations				●
Acute, sublethal	2. Algae (<i>Thalassiosira pseudonana</i>)	- Inhibition of population growth	- screening effluents - screening, monitoring effluents - screening of chemicals - selected investigations			○	○
	3. Intertidal clam (<i>Macoma balthica</i>)	- Bioaccumulation	- screening of contaminated sediments (ODCA)				
	4. Other clams (e.g. <i>Mya arenaria</i>)	- Behavior (burrowing, attachment)	- evaluation of contaminated sediments				
	5. Mussels (<i>Mytilus edulis</i>)	- Bioaccumulation	- evaluation of pesticide residues				○
	6. Flounder (<i>Pseudopleuronectes americanus</i>)	- MFO induction ⁴	- evaluation of marine toxicants				○

1 P = Pacific and Yukon;

W = Western and Northern;

Q = Quebec;

A = Atlantic

2 ● = frequently used test procedure

○ = occasionally used test procedure

TABLE 2 GENERAL TYPES OF AQUATIC TOXICITY TESTS USED IN ENVIRONMENTAL PROTECTION SERVICE LABORATORIES, AND EXTENT OF THEIR USE

Types of Tests	Testing System, in Phylogenetic Order									
	Ames test (Salmonella sp.)	Microtox test (Photobacterium)	Algae (Selenastrum)	Algae (Tholassiosira)	Protozoa (Colpidium)	Mollusca (Macoma, others)	Mollusca (Mytilus)	Crustacea (Daphnia)	Crustacea (Artemia)	Fish (Salmo gairdneri)
1. Lethal toxicity										
2. Sublethal toxicity										
Behavior - incapacitation										
- feeding inhibition										
- burrowing/attachment										
Physiological/biochemical stress										
Metabolic inhibition										
Growth										
Reproduction/Genetic										
3. Bioaccumulation										
● Frequent, routine test;										
○ Less frequent, often specialized test;										

TABLE 3 APPLICATION OF AQUATIC TOXICITY TESTS USED IN ENVIRONMENTAL PROTECTION SERVICE LABORATORIES

Testing System, in Phylogenetic Order		Ames test (Salmonella sp.)	Microtox test (Photobacterium)	Algae (Selenastrum)	Algae (Thalassiosira)	Protozoa (Colpidium)	Mollusca (Macoma, others)	Mollusca (Mytilus)	Crustacea (Daphnia)	Crustacea (Artemia)	Fish (Salmo gairdneri)	Marine Fish (Gasterosteus)	Marine Fish (other spp.)
Types of Tests													
1. Lethal toxicity				1,3,5,6		5	2,5	2,5	3,5,6	2,5	1,3,4,5,6,7	5,6	5,6
2. Sublethal toxicity													
Behavior - incapacitation													
- feeding inhibition													
- burrowing/attachment						5			3,5,6	2,5	1,3,4,5,6	5,6	5,6
Physiological/biochemical stress							2,5	2,5			5		5
Metabolic inhibition			1,3,5,6	1,3,5,6									
Growth				1,3,5,6	2,5								
Reproduction/Genetic	2,5,6								3,5,6		5		
3. Bioaccumulation				1,3,5,6			2,5	2,5			5		
Application-Legend													
Fisheries Act-regs., guidelines	1												
Ocean Dumping Control Act (ODCA)	2												
Contaminants Testing (OECD)	3												
EPS Dispersant Testing Guidelines	4												
Investigations of Selected Problems ^a	5												
Monitoring of Effluents	6												
Legal Tests	7												

a e.g. Chemicals from offshore activities, components of pesticide formulations, selected chemicals produced and transported in bulk.

APPENDIX A MAIN FEATURES OF THE TOXICITY TESTS USED IN THE EPS LABORATORIES, AS OF MAY 1984. TESTS ARE ORGANIZED BY REGION (Atlantic, Quebec, Western and Northern, and Pacific and Yukon). Data are presented as submitted to P.G. Wells, April-May, 1984, with minor corrections or editing.

EPS, ATLANTIC REGION

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organisms (2) Life Stage	Rainbow trout (<i>Salmo gairdneri</i>) Fryling (2.5-10g)
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	12 x 14°C, 96-hours duration. Static or flow-through. Lethality Rainbow trout are more sensitive than many fish, molluscs, and some crustaceans. Very high Within one lab = Very high (data available upon request)
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Tests (3) Training-Expertise	2 days test <1 day/day per static test. 2-3 week-days per flow-through. Minimal (adequate for flow-through)
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Excellent for rapid screening of effluents or effluents. Designed to assess influence of factors (e.g. pH) on the toxicity of a substance. Low requirements (space), rapid and simple to perform. Fresh water only. Ignores chronic and sub-lethal effects.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Basic References	See below.

1. Regulations and Guidelines for a variety of industrial sectors (e.g., Pulp and Paper, Oil refineries) issued by Environment Canada.
2. Standard procedure for testing the acute lethality of liquid effluents. Report EPS-1-WF-80-1, Environment Canada, Nov. 1980, 10p.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Rainbow trout (<u>Salmo gairdneri</u>) Fingerling (0.5-10g)
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	15 ± 1°C; 96-hours duration. Static or flow-through. Lethality Rainbow trout are more sensitive than many fish, molluscs, and some crustaceans. Very high Within one lab = Very high (data available upon request)
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	4 days test <1 Man-day per static test 2-3 man-days per flow-through Minimal (moderate for flow-through)
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Excellent for rapid screening of chemicals or effluents. Excellent to assess influence of factors (e.g. pH) on the toxicity of a substance (See usefulness above.) Rapid and simple to perform. Fresh water only. Ignores chronic and sub-lethal effects.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	See below.

- 1 Regulations and Guidelines for a variety of industrial sectors (e.g.: Pulp and Paper; Oil refineries) issued by Environment Canada.
- 2 Standard procedure for testing the acute lethality of liquid effluents. Report EPS-1-WP-80-1. Environment Canada. Nov. 1980. 10 p.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Rainbow trout (<u>Salmo gairdneri</u>) Fingerling (0.5-10g)
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	15 + 1°C; flow-through or static; various exposure periods Bioaccumulation of substances from test solutions Very low
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	Varies with duration of exposure. Includes exposures (3 to 10 or more man days) plus analysis of tissue samples. High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Has been used in EPS Atlantic region to determine uptake of fluoride by fish and uptake of aminocarb as influenced by pH. Bioaccumulation by a commercial species is important.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	None used.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Rainbow trout (<u>Salmo gairdneri</u>); Striped bass (<u>Morone saxatilis</u>) Egg/larvae
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Varies - depends on species requirements and exposure times. Flow through. Hatching success. Survival and growth of larvae. Probably quite sensitive. Little standardization. Not researched.
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	Time - consuming. Slow. High - perhaps >15 man-days per test High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Useful in measuring effects of toxicants on sensitive life stages of fish. Larval stages usually sensitive, as are sublethal parameters which are monitored. Time consuming & difficult to perform or repeat.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	See below.

- 1 Parker, W.R. and K.G. Doe. 1981. Environment Canada. Report: EPS-5-AR-81-6.
- 2 Ernst et al. 1980. Environment Canada. Report: EPS-5-AR-81-3.

1 Wong, S. 1982. Fish bioassay procedures for waste effluents from the APDA, CANADA, DOE, DFO, Report on effluents 411 & gas drilling fluid disposal in the Canadian North. July, 1982.

2 Doe, K.G., and P.G. White. 1978. In ASTM STP 639. Pages 15-17.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	3-spine stickleback (<u>Gasterosteus aculeatus</u>); Mummichog (<u>Fundulus heteroclitus</u>); Sandsmelt (<u>Menidia menidia</u>)
	(2) Life Stage:	Adult and sub-adult
(2) RESPONSES	(1) Test Conditions	4 days exposures (occasionally 10 days); 10 ± 1 or $15 \pm$ °C; static and occasionally flow-through.
	(2) Toxicological Criteria	Lethality.
	(3) Sensitivity	Comparable to other marine fish species in general.
	(4) Degree of Standardization	High for 3-spine stickleback (see reference 1 below).
	(5) Reproducibility	Good within one lab.
(3) PRACTICAL EVALUATION	(1) Rapidity	4 days.
	(2) Cost/Test	About 1 man day for static; 2-3 man days for flow-through test.
	(3) Training-Expertise	Minimal (moderate for flow-through)
(4) APPLICATION	(1) Usefulness	Good screening tool for large numbers of chemicals.
	(2) Major Advantage	Rapid and simple to perform. Marine species used.
	(3) Major Disadvantage	Ignores chronic and sub-lethal effects.
(5) BACKGROUND INFORMATION	(1) Documentation	See below.
	(2) Main References	

- 1 Wong, B. 1982. Fish bioassay procedure for waste drilling fluids. In: APOA, DIAND, DOE, DFO, Report on offshore oil & gas drilling fluid disposal in the Canadian North. July, 1982.
- 2 Doe, K.G., and P.G. Wells. 1978. In ASTM STP 659. Pages 50-65.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	Flounder (<u>Pseudopleuronectes americanus</u>)
	(2) Life Stage:	Adult
(2) RESPONSES	(1) Test Conditions	4 or more days exposure; ambient seawater temperature. Static. Aerated.
	(2) Toxicological Criteria	Lethality and some sub-lethal effects (e.g. MFO induction).
	(3) Sensitivity	
	(4) Degree of Standardization	No rigid test guidelines available.
	(5) Reproducibility	Unknown.
(3) PRACTICAL EVALUATION	(1) Rapidity	Depends on test duration.
	(2) Cost/Test	Minimum of 3 man-days per test due to large test volumes required.
	(3) Training-Expertise	Moderate.
(4) APPLICATION	(1) Usefulness	Marine commercial fish species - benthic in habitat - useful for drill cuttings work.
	(2) Major Advantage	In conjunction with DFO scientists, lethal and sublethal parameters were measured.
	(3) Major Disadvantage	
(5) BACKGROUND INFORMATION	(1) Documentation	
	(2) Main References	

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	Algae (<u>Selenastrum capricornutum</u> Printz)
	(2) Life Stage:	All
(2) RESPONSES	(1) Test Conditions	24 $\pm$ 2°C; 400 ft. candles; 14 days; Static - (shaken).
	(2) Toxicological Criteria	Growth.
	(3) Sensitivity	Low.
	(4) Degree of Standardization	High.
	(5) Reproducibility	Good.
(3) PRACTICAL EVALUATION	(1) Rapidity	14 days. Not a rapid test.
	(2) Cost/Test	3-4 man days.
	(3) Training-Expertise	Moderate.
(4) APPLICATION	(1) Usefulness	Uses sub-lethal parameter over several generations. Organisms = a primary producer.
	(2) Major Advantage	Uses a primary producer rather than an animal.
	(3) Major Disadvantage	Long test duration.
(5) BACKGROUND INFORMATION	(1) Documentation	See below.
	(2) Main References	
1	Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The <u>Selenastrum capricornutum</u> Printz algal assay bottle test. Experimental design, application, and data interpretation protocol. Office of Research and Development. USEPA. Corvallis, Oregon. 97330. USA. 126 pp.	
(3) BACKGROUND INFORMATION	(1) Documentation	Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The <u>Selenastrum capricornutum</u> Printz algal assay bottle test. Experimental design, application, and data interpretation protocol. Office of Research and Development. USEPA. Corvallis, Oregon. 97330. USA. 126 pp.
	(2) Main References	Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The <u>Selenastrum capricornutum</u> Printz algal assay bottle test. Experimental design, application, and data interpretation protocol. Office of Research and Development. USEPA. Corvallis, Oregon. 97330. USA. 126 pp.
1	Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The <u>Selenastrum capricornutum</u> Printz algal assay bottle test. Experimental design, application, and data interpretation protocol. Office of Research and Development. USEPA. Corvallis, Oregon. 97330. USA. 126 pp.	
2	Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The <u>Selenastrum capricornutum</u> Printz algal assay bottle test. Experimental design, application, and data interpretation protocol. Office of Research and Development. USEPA. Corvallis, Oregon. 97330. USA. 126 pp.	
3	Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The <u>Selenastrum capricornutum</u> Printz algal assay bottle test. Experimental design, application, and data interpretation protocol. Office of Research and Development. USEPA. Corvallis, Oregon. 97330. USA. 126 pp.	

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	Mussel (<u>Mytilus edulis</u>); soft-shelled clam (<u>Mya arenaria</u>); <u>Macoma balthica</u> ; freshwater clam (<u>Anodonta</u> sp.)
	(2) Life Stage:	Adult
(2) RESPONSES	(1) Test Conditions	Static or flow-through. Temperature variable (with season).
	(2) Toxicological Criteria	Lethal toxicity; sublethal effects (burrowing; attachment).
	(3) Sensitivity	Some sublethal effects, many times more sensitive than lethality.
	(4) Degree of Standardization	
	(5) Reproducibility	Unknown.
(3) PRACTICAL EVALUATION	(1) Rapidity	4-day tests with 4 or more days post-exposure in clean water.
	(2) Cost/Test	1 1/2-3 man days - depending on length of post exposure period.
	(3) Training-Expertise	Moderate.
(4) APPLICATION	(1) Usefulness	Sublethal effects on byssal attachment (mussels) or burrowing (<u>Mya</u> and <u>Macoma</u>) are very sensitive.
	(2) Major Advantage	Additional animal group besides fish. Benthic epi - or infauna, useful for pollutants impacting on sediments (e.g. drill cuttings).
	(3) Major Disadvantage	
(5) BACKGROUND INFORMATION	(1) Documentation	<u>Mytilus</u> and <u>Anodonta</u> tests described in Doe et al. 1983. Report EPS-5-AR-83-1.
	(2) Main References	See above, and also:

1 Roberts, D. 1975. Environ. Pollut. 8: 241-254.

2 Martella, T. 1974. Water, Air and Soil Pollut. 3:171-177.

3 See above under (1) Documentation.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Macoma balthica</u> Adult
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	30-35 days. Static. $15 \pm 1^\circ\text{C}$. Aerated. Bioaccumulation of cadmium or PCB from sediment.
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	30-35 days minimum duration. Requires 5-7 days for exposures & analyses on tissue samples. Moderate.
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Used for ODCA investigations. Simplicity. In addition, of the species screened, <u>Macoma</u> was best able to bioaccumulate cadmium from sediment.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Various unpublished EPS reports on contaminated sediments.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Mussel (<u>Mytilus edulis</u>) Adult (1" to 3" shell length)
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Flow-through. 30 days exposure + 10 days depuration. Bioaccumulation of pesticide from test solution. High (see main reference ² below). Unknown - but should be repeatable using "standard" method.
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	40 days total time (not rapid). 10 or more man-days for exposure in addition to analysis of tissue samples. High.
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Excellent bioaccumulation test for an important species (ecologically and commercially). Could be used to establish uptake/depuration rates as well as bio-concentration factor. Expensive & time - consuming.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	See below.

1 Doe, et al., 1983. Report EPS-5-AR-83-1.

2 Anonymous. 1980. Degradability, ecotoxicity and bio-accumulation. Government Publishing Office, The Hague, The Netherlands.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Artemia salina</u> (brine shrimp) Larvae (instar II-III nauplii)
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	24 hour; static; artificial dilution water (seawater); $25 \pm 1^\circ\text{C}$; incubate in darkness. Lethality. Not as sensitive as trout or <u>Daphnia</u> to DSS or Chromium. Very high (see reference 1). Good with one lab. Should be reasonable between labs.
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	Very rapid. Approx. or less than 1 man-day/test Minimal.
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Rapid screening. Can be used world-wide using some strain of test organisms a same dilution water (intercomparable). Ignores chronic and sublethal effects.
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	See below.

- 1 Vanhaecke, et al, 1981. Proposal for a short-term toxicity test with Artemia nauplii.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	<u>Daphnia pulex</u> and <u>Daphnia magna</u> (water fleas)
	(2) Life Stage:	Young (usually <2 days old)
(2) RESPONSES	(1) Test Conditions	22 ± 2°C; 48 hour exposures; static; unaerated; 2 animals/50 ml test solution.
	(2) Toxicological Criteria	Lethality.
	(3) Sensitivity	Generally more sensitive than most fish species.
	(4) Degree of Standardization	Fairly high - see reference 1.
	(5) Reproducibility	Within a lab = fair. Low or moderate between labs. (See reference 1.)
(3) PRACTICAL EVALUATION	(1) Rapidity	Very rapid. 2 days.
	(2) Cost/Test	1 man-day/test.
	(3) Training-Expertise	Moderate - more complex than rainbow trout test.
(4) APPLICATION	(1) Usefulness	Rapid screening tool. Also useful where test substance is present in very limited quantities.
	(2) Major Advantage	Rapid; low volumes of test materials; small space requirement.
	(3) Major Disadvantage	More complex than trout test. Control mortality sometimes a problem.
(5) BACKGROUND INFORMATION	(1) Documentation	See below.
	(2) Main References	

- 1 Parker, W.R. M.S. 1983. Results of the interlaboratory study on the toxicity of potassium dichromate to Daphnia. July, 1983. (unpublished report).

APPENDIX B MAIN FEATURES OF TEST IN THE LABORATORY

	Feature	Description
(1) TEST	(1) Objective	Reviews from before and after
	(2) Data Source	Fingerprint
(2) RESEARCHERS	(1) Test Conditions	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(2) Test Conditions	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(3) Test Conditions	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(4) Test Conditions	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(5) Test Conditions	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(6) Test Conditions	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
(3) PRACTICAL EVALUATION	(1) Application	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(2) Application	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(3) Application	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(4) Application	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
(4) APPLICATION	(1) Application	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS
	(2) Application	L - Start of Cap-Sa test 15 min before APL - Start of CS - Start of CS

EPS, QUEBEC REGION

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

Features		Description
(1) TEST	(1) Organism: (2) Life Stage:	Rainbow trout <u>Salmo gairdneri</u> Fingerling
(2) RESPONSES	(1) Test Conditions	L - Static LC ₅₀₋₉₆ hrs 15 test basins, ASL - Static, CSL - Semi Static
	(2) Toxicological Criteria	L - mortality evaluation after 4 day-exposure, ASL - physiological stress (ATP in muscle tissue) after 4 day exp., CSL - physiological stress (ATP in muscle tissue) after 21 day exp.
	(3) Sensitivity	L good; ASL good; CSL good
	(4) Degree of Standardization	L very good; ASL good; CSL good
	(5) Reproducibility (intra) (inter)	L good; ASL good; CSL unknown L good; ASL unknown; CSL unknown
(3) PRACTICAL EVALUATION	(1) Rapidity	L moderately fast - 4d; ASL mod. fast - 4d; SCL slow - 21d
	(2) Effort/Test	L 2.5 man day/test; ASL 1.5 man day/test; CSL 3.5 man day/test
	(3) Cost/Test	L \$650.; ASL \$650.*; CSL \$1200.*
	(4) Training-Expertise	L Medium; ASL High; CSL High
(4) APPLICATION	(1) Usefulness	L - High, monitoring effluents chemicals and selected investigations; ASL - High as above; CSL - High but mostly for selected investigations
	(2) Major Advantage	L - Internationally recognized and standardized easy to demonstrate ecotox. significance; ASL - As a complement to above, increases sensitivity economically; CSL - As a complement to above, characterizes chronic effect

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

Features		Description
(3) Major Disadvantage		L - Large sample size and space requirements - Labor intensive; ASL - as above and requires highly trained personnel; CSL - as above and long duration 21 days
(5) BACKGROUND INFORMATION	(1) Documentation	L Significant; ASL Rare; CSL nonexistent
	(2) Main References	ASL Blaise 83; CSL to be published 1984-85
L	Lethal	Very good
ASL	Acute Sublethal	Very good
MTSL	Mid Term Sublethal	Good
CSL	Chronic Sublethal	Good
(4) APPLICATION		(1) Uncertain
		(2) Major Advantage
		(3) Major Disadvantage
(5) BACKGROUND INFORMATION		(1) Documentation
		(2) Main References
L	Lethal	Very good
ASL	Acute Sublethal	Very good
MTSL	Mid Term Sublethal	Good
CSL	Chronic Sublethal	Good

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Brine Shrimp <u>Artemia nauplii</u> Larval
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility (intra) (inter)	Static L mortality (as exhibited by the immobilization of the organism after 1 day exposure) Good High Very good Very good
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Effort/Test (3) Cost/Test (4) Training-Expertise	Fast, 24 hours 5 man day/10 tests \$150 Low
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	High, screening for monitoring effluents, chemicals, and in selected investigations Internationally recognized standard, practical and economical Relatively good potential for automation Ecological significance relatively difficult to demonstrate
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Significant Personne, G., Ghent, Belgium.

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Protozoans <u>Colpidium campylum</u> Mature cells undergoing division
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility (intra) (inter)	Static L mortality (as exhibited by the organism inhibition to ingest and clarify a turbid <u>E. coli</u> food source after 1 day exposure) Good Good Good Unknown
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Effort/Test (3) Cost/Test (4) Training-Expertise	Fast, 24 hours 2 man day/10 tests \$50.* Medium
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	High, screening for monitoring effluents, chemicals and selected investigations Highly economical and fast High potential for automation Comparative data wanting
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Limited Dive, D. (France).

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	Diatomaceous algae <u>Thalassiosira pseudonana</u>
	(2) Life Stage:	Mature cells undergoing division
(2) RESPONSES	(1) Test Conditions	Static
	(2) Toxicological Criteria	MTSL inhibition of growth
	(3) Sensitivity	High
	(4) Degree of Standardization	Good
	(5) Reproducibility (intra) (inter)	Good Unknown
(3) PRACTICAL EVALUATION	(1) Rapidity	Moderately fast - 4 days
	(2) Effort/Test	2 man day/10 tests
	(3) Cost/Test	\$50.
	(4) Training-Expertise	Medium high
(4) APPLICATION	(1) Usefulness	High, screening for monitoring effluent chemicals, and in selected investigations
	(2) Major Advantage	Relatively fast, economical and significant in response High potential for automation
	(3) Major Disadvantage	Comparative data lacking
(5) BACKGROUND INFORMATION	(1) Documentation	Limited
	(2) Main References	Sabatini (1983)

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	Green algae <u>Selenastrum capricornutum</u>
	(2) Life Stage:	Mature cells undergoing division
(2) RESPONSES	(1) Test Conditions	ASL - static; MTSL - static; AB - static
	(2) Toxicological Criteria	ASL - physiological stress (ATP in after 1 day exposure (occasional) MTSL - inhibition of population growth after 4 day exposure (routine); AB - bioaccumulation after 4 h to 24 h exposure (routine)
	(3) Sensitivity	ASL - very good; MTSL - high; AB - good
	(4) Degree of Standardization	ASL - good; MTSL - very good; AB - fair
	(5) Reproducibility (intra) (inter)	ASL good; MTSL - good; AB - fair ASL unknown; MTSL - good; AB - unknown
(3) PRACTICAL EVALUATION	(1) Rapidity	ASL - fast 4h to 24h; MTSL - mod. fast 4 days; AB - fast 4h to 24h
	(2) Effort/Test	ASL - 3 man days/10 tests; MTSL - 1.5 man day/10 tests; AB - 2 man days/10 tests
	(3) Cost/Test	ASL - \$150.*; MTSL - \$50.; AB - \$150.*
	(4) Training-Expertise	ASL High; MTSL Medium; AB - High
(4) APPLICATION	(1) Usefulness	ASL - High, mainly for selected investigations and rapid screening test; MTSL - High, screening for monitoring effluent, chemicals, and for selected investigations; AB - High, same as for MTSL

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

Features		Description
	(2) Major Advantage	ASL - Extremely rapid screening tool and with MTSL helps predict dynamism in toxic response MTSL - Relatively fast sensitive and economic, high potential for automation; AB - Only economical and practical tool available to predict bioaccumulation
	(3) Major Disadvantage	ASL - Same as MTSL and comparative data wanting; MTSL - Applicable only to sample pre-filtered at 0.22u; AB - Same as MTSL and comparative data wanting
(5) BACKGROUND INFORMATION	(1) Documentation	ASL - fair; MTSL - significant; AB - rare
	(2) Main References	Vasseur 80; R. Van Coillie 82; AB Blaise 83

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Strains <u>Salmonella typhimurium</u> Mature cell growth
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility (intra) (inter)	Static CSL - Mutagenesis (AMES - fluctuation test) High Very good Good Good
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Effort/Test (3) Cost/Test (4) Training-Expertise	Moderately fast - 4 days 4 man day/10 tests \$150.* High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	High, screening for monitoring effluents, chemicals and in selected investigations The most economical practical and reliable tool to predict mutagenesis to date High potential for automation Cautious interpretation of results is necessary to avoid alarmist consequences
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Significant AMES

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Photobacterium phosphoreum</u> Cell metabolism
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility (intra) (inter)	Static ASL - inhibition of light emission Good High Very good Very good
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Effort/Test (3) Cost/Test (4) Training-Expertise	Very fast, 5 to 15 minutes response 2 man day/10 tests \$100. Medium
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	High, screening for monitoring effluents, chemicals and selected investigations Fast, economical and reliable small volume required permits application of toxic response to various selected investigation Ecotoxicological significance difficult to demonstrate
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Significant Bulich 80

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism:	BDC Plus Type H1 <u>Dried Bacterial Cultures</u>
	(2) Life Stage:	Active cell growth
(2) RESPONSES	(1) Test Conditions	Static aerated test basins. Samples for ecotox evaluations are subjected for 5 days to regenerated acclimated bacterial cell cultures
	(2) Toxicological Criteria	Biodegradability step to predict persistence of toxic response - Biodegradation test
	(3) Sensitivity	Good
	(4) Degree of Standardization	Low
	(5) Reproducibility (intra) (inter)	Good Unknown
(3) PRACTICAL EVALUATION	(1) Rapidity	Moderately fast - 5 days
	(2) Effort/Test	1 man day/10 tests
	(3) Cost/Test	\$50.*
	(4) Training-Expertise	Low
(4) APPLICATION	(1) Usefulness	High, screening for monitoring effluents, chemicals and selected investigations
	(2) Major Advantage	The only practical and economical tool available to predict persistence of toxic effects
	(3) Major Disadvantage	In house methodology applied only
(5) BACKGROUND INFORMATION	(1) Documentation	Rare
	(2) Main References	In house procedure

L Lethal
 ASL Acute Sublethal
 MTSL Mid Term Sublethal
 CSL Chronic Sublethal

APPENDIX A: MAIN FEATURES OF EPS IN THE LABORATORY

	Features	Description
(1) EPS	(1) Overview	Being planned
	(2) Life Cycle	Lowest
(2) RESEARCH	(1) Test Conditions	State of Equipment is Flow enough
	(2) Technological Criteria	Variability
	(3) Reliability	Medium to High
	(4) Degree of Standardization	High
	(5) Standardization	High
(3) PRACTICAL EVALUATION	(1) Factors	4-5%
	(2) Cost/Time	Excluding capital & material approximately 10000%
	(3) Training Features	Medium
(4) APPLICATION	EPS, WESTERN AND NORTHERN REGION	
	(1) Major Advantages	The test is universally applicable
	(2) Major Disadvantages	Lab. capacity
(5) BACKGROUND INFORMATION	(1) Documentation	Excluding Technicalities and Standardization
	(2) Main References	EP's Standard Methods ASTM, ISO, V, Standard Methods

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Salmo gairdneri</u> Juvenile
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Static or Replacement or Flow through Mortality Medium to High High High
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	4 days Excluding capital & salaries: approximately \$200.00 Medium
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Good The test is universally acceptable Labor intensive
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Exceptional documentation and standardization EPS Standard Method, ASTM, OECD, Standard Methods

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Daphnia magna</u> 0-48 hours
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Static or Replacement Mortality or incapacitation High Medium to High High
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	2-4 days <\$20.00 Medium
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Good Quick, and requires small sample sizes Labor intensive, particularly maintenance of stocks
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Very good in normal literature EPS Standard Method; ASTM Standard Methods

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Selenastrum capricornutum</u> Normal Log growth
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Constant illumination/temperature; static Population growth/cell counts High High High
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	7-8 days \$50.00 Medium High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Good Small sample size; automation; high accuracy Capital intensive; labor intensive
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Good EPS Standard Algal Assay Method; OECD Method

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Gasterosteus aculeatus</u> <1 year old
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Static Mortality Medium to High High High
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	4 days Approx. \$300.00 Medium
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Good Allows marine testing of samples Poor availability of test organisms
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Poor to Fair EPS Standard Method

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Microtox ^R (<u>Photobacterium</u> sp.) Lyophilized Bacteria
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Temperature controlled photometer Metabolic effects monitored by luminescence High High High
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	Extremely rapid (5 minutes - 30 minutes) \$50.00 Medium-Medium High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Good Extremely rapid High capital outlay; non-realistic to actual environment
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Good Beckman Instruments Inc.

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	Rainbow Trout (<u>Salmo gairdneri</u>) Juvenile
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Replacement: Flow through Mortality High Medium to High High
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	Long Term Exposure - Open Time Frame >\$200.00 Medium - Medium High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Good Gives more realistic information; more sensitive Time consuming; labour and sample intensive
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Few methods available Wells and Moyse (1981).

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST	(1) Organism: (2) Life Stage:	<u>Daphnia magna</u> 0-24 Hrs.
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Replacement Mortality and reproduction High Medium Good
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	Slow 2-3 weeks \$20-100.00 Medium High to High
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Very Gives lethal and sublethal data Extremely labour intensive; requires good techniques from operators
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Fair to Good ASTM Draft Procedures

APPENDIX A MAIN FEATURES OF TEXT IN THE LANCASHIRE

	Feature	Description
(1) TEST FROM LANCASHIRE UNIVERSITY	(1) Organisation (2) Life Stage	Early Day (Early Childhood) Pre-linguistic Development
(2) RESPONSES	(1) Test Condition (2) Topic/Stage/Theme (3) Stimulus (4) Degree of Structuration (5) Repetition	Early Day (Early Childhood) Narrative, Topic/Stage/Theme High security Structured 1-2 Day Cycle/Stage/Theme
(3) FROM THE LANCASHIRE	(1) Register (2) Test/Tax (3) Preparing/Preparing	1-2 Day Early Day (Early Childhood) - 1-2 Day Early Day (Early Childhood)
(4) APPLICATION	(1) Early Day (2) Early Advantage (3) Early Disadvantage	Early Day (Early Childhood) Early Day (Early Childhood) Early Day (Early Childhood)
(5) EXPLORING THE LANCASHIRE	(1) Description (2) Main Features	Early Day (Early Childhood) Early Day (Early Childhood)

EPS, PACIFIC REGION

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST Acute Lethal -freshwater	(1) Organism: (2) Life Stage:	Rainbow Trout (<u>Salmo gairdneri</u>) Fry-fingerling (underyearling)
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Static/flow-through Mortality, Incapacitation High sensitivity Standardized $\pm 5\%$ (see Craig-Interlab Cu study)
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	≤ 96 hours static $\approx \$200$, flow-through $\approx \$1,500$ Biology, plus >1 yr experience
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Screening, Regulatory/compliance Rapid response, low cost Depending on toxicant-can require large volumes of material for test
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	EPS 1-WP-80-1, P&Y Lab. Man.(79) ASTM STP 528(73), Std. Methods 15th ed.(80)

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST Microtox	(1) Organism: (2) Life Stage:	<u>Photobacterium phosphoreum</u> n/a
(2) RESPONSES	(1) Test Conditions (2) Toxicological Criteria (3) Sensitivity (4) Degree of Standardization (5) Reproducibility	Static - EC50 Change in light output In most cases similar to R.T. Standardized (photometrically) ± 3%
(3) PRACTICAL EVALUATION	(1) Rapidity (2) Cost/Test (3) Training-Expertise	20 - 30 minutes \$30- \$40 Biology + chemistry
(4) APPLICATION	(1) Usefulness (2) Major Advantage (3) Major Disadvantage	Screening, some monitoring Fast results, transportable, small sample volumes required (<1 litre.) Adjustment of sample to 2% NaCl
(5) BACKGROUND INFORMATION	(1) Documentation (2) Main References	Beckman manual no. 110679, ASTM STP 667(79) ISA Trans., vol 20, no. 1(81) ASTM STP 667 pp68-106, 1979

APPENDIX A MAIN FEATURES OF TEST IN THE LABORATORY

	Features	Description
(1) TEST Acute lethal -saltwater	(1) Organism:	3 spined Stickleback (<u>Gasterosteus aculeatus</u>)
	(2) Life Stage:	2-4 cm
(2) RESPONSES	(1) Test Conditions	Static
	(2) Toxicological Criteria	Mortality, Incapacitation
	(3) Sensitivity	Moderate
	(4) Degree of Standardization	Nil
	(5) Reproducibility	Perhaps $\pm$ 10%
(3) PRACTICAL EVALUATION	(1) Rapidity	$\leq$ 96 hours
	(2) Cost/Test	as Trout
	(3) Training-Expertise	as Trout
(4) APPLICATION	(1) Usefulness	Screening, Monitoring
	(2) Major Advantage	as Trout
	(3) Major Disadvantage	Somewhat plastic in response
(5) BACKGROUND INFORMATION	(1) Documentation	
	(2) Main References	Standard Methods 15th ed. (80)

